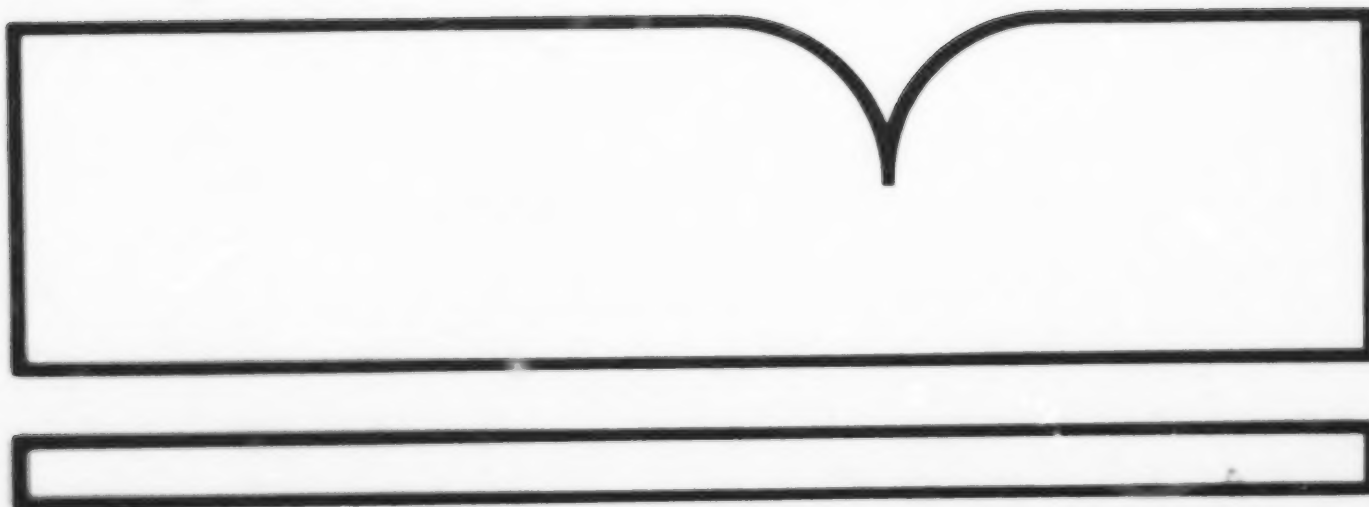


PB87-116646

Effects of Metal Salt Mixtures on
'Daphnia magna' Reproduction

(U.S.) Environmental Research Lab.-Duluth, MN

1986



U.S. Department of Commerce
National Technical Information Service

NTIS

TECHNICAL REPORT DATA (Please read instructions on the reverse before comg)		
1. REPORT NO. EPA/600/J-86/142	2.	PB87-116646
4. TITLE AND SUBTITLE Effects of Metal Salt Mixtures on <u>Daphnia magna</u> Reproduction. (Journal Version)	5. REPORT DATE 1986	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) K.E. Biesinger, G.M. Christensen, and J.T. Fiandt	8. PERFORMING ORGANIZATION REPORT NO.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, MN 55804	10. PROGRAM ELEMENT NO.	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Duluth, MN 55804	13. TYPE OF REPORT AND PERIOD COVERED	14. SPONSORING AGENCY CODE EPA-600/03
15. SUPPLEMENTARY NOTES Published in: Ecotoxicology and Environmental Safety 11, pp. 9-14, 1986. (February).		
16. ABSTRACT Three binary metal experiments were conducted using a "complete block design"; testing the chlorides of Cd, Hg, and Zn individually and in combinations of Cd-Hg, Cd-Zn, and Zn-Hg on <i>Daphnia magna</i> reproduction. These mixtures were tested at one-half, once, and twice the 16% reproductive impairment concentration previously determined for individual metals. The Cd-Hg, Cd-Zn, and Zn-Hg mixtures all showed significant reductions in reproduction at concentrations where the metal salts alone caused no significant effect.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT Release to public.	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 7
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

Effects of Metal Salt Mixtures on *Daphnia magna* Reproduction

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Received July 15, 1985

Three binary metal experiments were conducted using a "complete block design"; testing the chlorides of Cd, Hg, and Zn individually and in combinations of Cd-Hg, Cd-Zn, and Zn-Hg on *Daphnia magna* reproduction. These mixtures were tested at one-half, once, and twice the 16% reproductive impairment concentration previously determined for individual metals. The Cd-Hg, Cd-Zn, and Zn-Hg mixtures all showed significant reductions in reproduction at concentrations where the metal salts alone caused no significant effect. © 1986 Academic Press, Inc.

INTRODUCTION

Several studies have been conducted to determine the acute effects of mixtures of inorganic salts and other pollutant mixtures to aquatic life, but few chronic studies have been completed.

The European Inland Fisheries Advisory Commission (1980) reviewed principles proposed for studying the joint effects of toxicants, including models and terminology, and summarized much of the literature concerning joint action of mixtures of chemicals to aquatic organisms. They suggested using the "concentration addition" model for water pollution control as proposed by Anderson and Weber (1975) for acute studies of toxicant mixtures. The few reported chronic studies on fish of mixtures of chemicals (Eaton, 1973; Muska, 1978; Broderius and Smith, 1979; and Spehar *et al.*, 1978) show somewhat conflicting results. Gray and Ventilla (1973) found zinc, lead, and mercury in various combinations to depress the growth rate of a marine protozoan; they found the effects to be more or less than additive depending on the concentration tested.

The present study was conducted to determine the effects of relatively toxic metal salt mixtures on *Daphnia magna*, using reproductive success as the endpoint. The three preparations were binary mixtures tested in a complete block design at one-half, once and twice a previously determined 16% reproductive impairment (a nonsignificant effect) concentration (Biesinger and Christensen, 1972).

METHODS

D. magna were cultured at the Environmental Research Laboratory—Duluth. The renewed static test procedure described by Biesinger and Christensen (1972) was followed. For each test four replicate test chambers (250-ml beakers with 200 ml of solution) each containing five daphnids for each experimental treatment and control

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were used. Three tests were performed with mixture 3 and two tests with mixtures 1 and 2. Daphnids in test chambers were randomly placed in a water bath and maintained at $18 \pm 1^\circ\text{C}$. They were transferred into fresh media after the first and second week of exposure. Young were counted and discarded when the media was changed and at the end of the 3-week experiments. Fluorescent bulbs provided 115 L of light at the air water interface with a 16-hr photoperiod. The animals were fed a mixture of trout food and dried grass.

Lake Superior water used for these tests had a pH of 7.4–8.2, a total hardness of 45 ± 1.26 mg/liter (as CaCO_3), and an alkalinity of 42 ± 1.19 mg/liter (as CaCO_3). The metal salt mixtures did not change these parameters. Additional chemical characteristics of this water have been published (Biesinger and Christensen, 1972).

Mixture 1, 2, and 3 experiments were conducted using a "complete block design" involving testing each of three individual chemicals and all possible binary combinations. These experiments were conducted by testing mixtures at the 16% reproductive impairment concentration for each metal, and at one-half and twice these values.

The results of these tests are presented in terms of reproductive success rather than impairment; they are all presented as normalized to controls, which are considered to have 100% reproductive success.

Reagent grade chemicals used for testing were HgCl_2 , $\text{CdCl}_2 \cdot 2\frac{1}{2} \text{H}_2\text{O}$ and ZnCl_2 . Concentrated stock solutions were prepared for each individual metal and diluted to the final stock solutions in lake water the day tests were started or renewed. Metals were added to ~950 ml of water individually for each test and concentration. The solutions were stirred between each addition and then made up to a liter with food and lake water. Analytical measurements of the solutions were made. Mercury was analyzed by flameless atomic absorption spectrophotometry as described by Olson *et al.* (1975). Precision and accuracy were checked by adding 1.00 and 0.10 μg Hg/liter to the control water followed by analysis to determine the recovery. Recovery from water samples and reagent blanks was determined each time samples were analyzed and was $96.6 \pm 6.7\%$.

Cadmium and zinc samples were acidified with concentrated HNO_3 to a pH of 2, and analyzed by atomic absorption spectrophotometry. The instrument was equipped with an air-acetylene flame for the zinc analysis and a graphite tube furnace for cadmium. The methods were verified using preanalyzed samples from the EPA Quality Assurance Laboratory in Cincinnati. For the standard zinc sample, whose true concentration was 79 μg /liter, the analysis gave a concentration of 78 ± 5 μg /liter ($N=53$). For cadmium, with a true concentration of 1.8 μg /liter, the analysis showed a concentration of 1.9 ± 0.2 μg /liter ($N=9$).

Added and analyzed measurements of metals for the binary tests are given in Table 1. Added and measured cadmium and zinc concentrations are approximately the same; however, measured mercury concentrations showed a decline with time. An exponential decay formula, as given in Biesinger *et al.* (1982), was used to obtain an average concentration with time.

Statistical analyses. Effects on production of young were evaluated using a $\sqrt{x} + \sqrt{x+1}$ transformation (x = number of young produced per beaker), a two-way analysis of variance and Dunnett's Test ($P \leq 0.05$) (Steel and Torrie, 1960). As experiments were designed to show effects on production of young, most tests were not conducted at concentrations high enough to show lethal effects to the parent generation.

TABLE I
METAL CONCENTRATIONS IN BINARY METAL SALT MIXTURES

Metal ion	Added concentration ($\mu\text{g/l}$)	Mean analyzed water concentration ($\mu\text{g/l} \pm$ standard deviation)	Number of measurements
Cadmium	0.09	0.09 ± 0.03	10
	0.17	0.19 ± 0.03	21
	0.34	0.35 ± 0.08	19
Mercury	1.70	0.91 ± 0.07	12
	3.40	1.82 ± 0.18	12
	6.80	3.53 ± 0.29	16
Zinc	35.00	37.90 ± 10.80	16
	70.00	74.00 ± 9.50	35
	140.00	140.30 ± 11.40	35

RESULTS

In binary mixture tests (Figs. 1, 2, and 3), there were significant decreases ($P \leq 0.05$) in reproductive success by daphnids when metal salts were combined at concentrations which did not significantly decrease reproductive success when the metal salts were tested individually. In the Cd-Hg mixture Hg did not significantly affect reproduction at a concentration of 3.5 $\mu\text{g/liter}$ Hg; Cd did not significantly affect reproduction at concentrations of 0.09, 0.19, or 0.35 $\mu\text{g/liter}$; however, when Hg and Cd were tested together at these concentrations there were significant effects on reproduction.

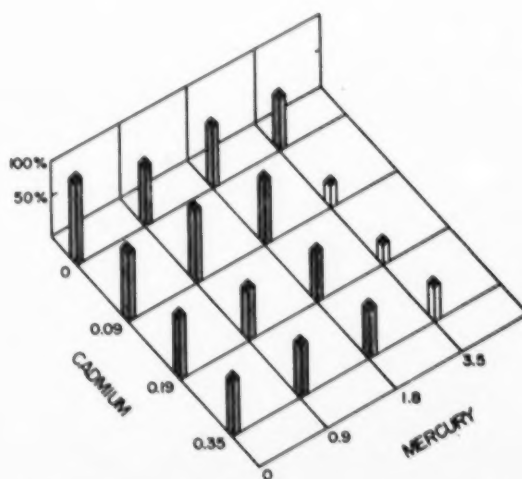


FIG. 1. Reproductive success of *Daphnia magna* exposed to a binary mixture of cadmium and mercury. Light bars indicate that reproduction is significantly less ($P \leq 0.05$) than controls. (Concentrations are in $\mu\text{g/l}$).

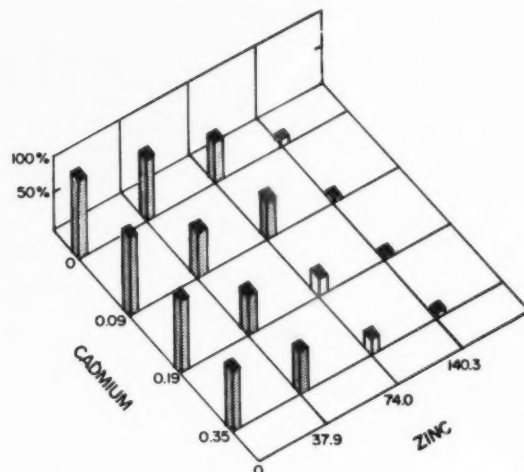


FIG. 2. Reproductive success of *Daphnia magna* exposed to a binary mixture of cadmium and zinc. Light bars indicate that reproduction is significantly less ($P \leq 0.05$) than controls. (Concentrations are in $\mu\text{g/l}$).

In the Cd-Zn mixture Cd alone did not significantly affect reproduction at any of the concentrations tested. However, when 74 $\mu\text{g/liter}$ Zn were added with Cd at concentrations of 0.19 and 0.35 $\mu\text{g/liter}$ there were significant effects on reproduction. Zinc at a concentration of 140.3 $\mu\text{g/liter}$ affected reproduction alone and with all concentrations of Cd tested.

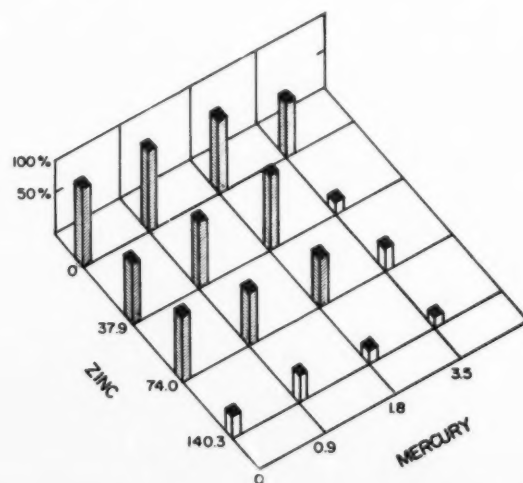


FIG. 3. Reproductive success of *Daphnia magna* exposed to a binary mixture of zinc and mercury. Light bars indicate that reproduction is significantly less ($P \leq 0.05$) than controls. (Concentrations are in $\mu\text{g/l}$).

In the Zn-Hg tests Zn caused significant effects at a concentration of 140.3 $\mu\text{g/liter}$ alone and with all concentrations of Hg. Significant effects of the Hg-Zn mixtures were found at a Hg concentration of 3.5 $\mu\text{g/liter}$ and Zn concentrations of 37.9 and 74.0 $\mu\text{g/liter}$ at concentrations not found when the metals were tested alone.

DISCUSSION

The results from all tests suggest that metals combined at no effect concentrations of the individually tested metals significantly affected reproduction of *D. magna* which indicated that toxicity was enhanced by interactions on a chronic basis. This correlates with the theoretical proposition that the soluble transition metal cations as a group may have a colligative action especially with biochemical sulfhydryl functional groups possessed by many enzymes and other macromolecules that control metabolism, whereby interference with these rate-controlling factors may be directly involved in toxicogenesis. Much additional work is needed in order to understand the mode of action of toxic inorganic and organic pollutants, acting singly and in combination. Further studies are needed to know precisely how the toxicants are absorbed, which biochemicals are target sites, what are the chemical characteristics of the receptors, and to better understand the characteristics of biotransformation, biodegradation, and elimination.

Water quality criteria for protecting aquatic organisms are based on single pollutants and multiple genera of aquatic life (USEPA, 1985), but do not deal with multiple pollutants which occur in almost all discharges and natural waters. The results of the present study and also the limited number of chronic studies by others suggest the limitations of single pollutant criteria. Spehar and Fiandt (1985) tested arsenic, cadmium, chromium, copper, mercury, and lead at criterion average concentrations and found a significant reduction in reproduction by ceriodaphnids (*Ceriodaphnia dubia*) and in growth with fathead minnows (*Pimephales promelas*); however, rainbow trout (*Salmo gairdneri*) were not significantly affected. Chronic tests they conducted at the maximum acceptable toxicant concentration (MATC), as determined from tests with each species, showed that joint action was somewhat less than additive to fathead minnows but nearly strictly additive to ceriodaphnids. The authors suggested that long-term metal interactions may differ in fish and invertebrates. However, it is evident that criteria should be established to protect all aquatic life and that the present criteria will not do this for mixtures. The Environmental Protection Agency has made some allowance for mixtures of pollutants by developing a whole effluent approach as the complexity of mixtures is at best poorly understood. It is evident that this is an area which needs to be studied in much greater depth and that water quality criteria should have provisions for dealing with mixtures of chemicals that have similar chemical and physical properties and should not be based exclusively on the "no observed effect" concentrations of individual chemicals even when that data is based upon chronic testing.

CONCLUSIONS

Binary mixtures of three metal salts caused significant effects on reproduction of daphnids at concentrations which did not significantly affect young production when the metals were tested individually. The results show that mixtures combined at es-

entially "no effect" concentrations for individual toxicants still exert toxic action in chronic tests as well as acute tests and suggest that water quality criteria and standards based on these endpoints may not be adequate to protect some aquatic species when mixtures of toxicants are present. A provision is needed for each water quality criteria stating that other pollutants at "safe levels" may affect the toxicity of individual toxicants and cause adverse sublethal effects with aquatic life.

ACKNOWLEDGMENTS

The authors sincerely thank A. Shelton for her assistance with the bioassays, B. Halligan for drawing the figures, and S. Heintz and T. Highland for typing the manuscript.

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